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A NOVEL BIOSYNTHETIC ROUTE TO PREGNANES IN THE MARINE SPONGE *AMPHIMEDON COMPRESSA*

RUSSELL G. KERR,* KATHLEEN KELLY, and AARON SCHULMAN

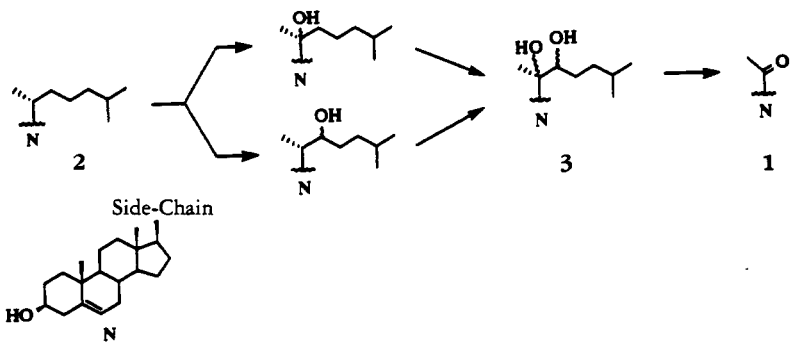
Department of Chemistry, Florida Atlantic University, Boca Raton, Florida 33431

ABSTRACT.—A novel degradative route to pregnanes has been elucidated in the marine sponge *Amphimedon compressa*. 22-Dehydrosterols, common in the diet of sponges, are the precursors to pregnanes and apparently are degraded by a mechanism analogous to the dealkylation of phytosterols to cholesterol.

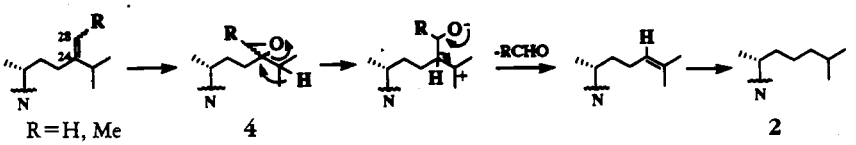
A number of novel pregnanes have recently been isolated from marine invertebrates including sponges, coelenterates and echinoderms (1,2). We report here documentation of a novel degradative pathway leading to the production of such steroids in the sponge *Amphimedon compressa* (=*Haliclona rubens*, Niphatidae, Duchassaing & Michelotti, 1864). This mechanism is analogous to the dealkylation of phytosterols to cholesterol known to operate in marine sponges (3).

The metabolic role of pregnanes in higher animals has been studied in great detail (4). Pregnenolone [**1**] is an intermediate in the production of progesterone from cholesterol [**2**], involving the intermediacy of 20,22-dihydroxycholesterol [**3**] (Scheme 1). However, in light of our mechanistic work (3) concerning the dealkylation of phytosterols with $\Delta^{24(28)}$ unsaturation (Scheme 2), it occurred to us that an analogous pathway could account for the conversion of Δ^{22}-sterols to pregnanes (Scheme 3). Both classes of sterols are common in the diet of marine sponges (2).

Key features of the dealkylation of phytosterols to cholesterol include the production of a 24,28-epoxide [**4**], and the migration of a hydride from a tertiary center to C-24 with concurrent opening of the epoxide (Scheme 2). Δ^{22}-Sterols [**5**] contain at least one tertiary carbon α- to epoxide carbons, and thus analogous hydride migrations could result in the opening of epoxide **6**, which could lead to the cleavage of the C-22–C-23 bond. Loss of the C-22 methylene could proceed in a manner similar to that in the degradation of 24-methylenecholesterol. Several of the intermediates proposed in Scheme 3 are known marine metabolites. Sterols with a 22(23)-epoxide moiety have been isolated from several echinoderms (5,6), as well as the sponge *Hyrtius* sp. (7). Aldehyde **7** was also isolated from this sponge (7), and 20-methylenepregnane [**8**] co-occurs with pregnenolone in the sponge *Damiriana hawaiiana* (8).



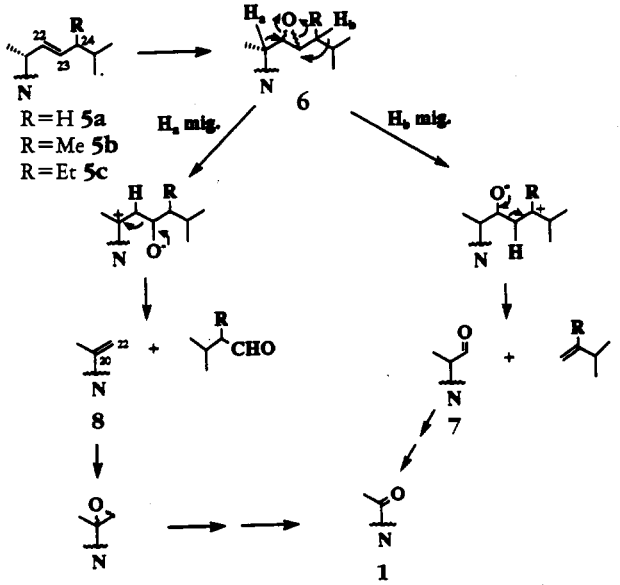
SCHEME 1. Transformation of cholesterol to pregnenolone [**1**].



SCHEME 2. Degradation of C-24 alkyl groups in sterols.

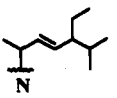
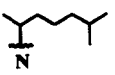
To determine the biosynthetic origin of pregnanes in *A. compressa,* radiolabeled cholesterol [**2**], stigmasterol [**5c**], and 20-methylenepregnane [**8**] were administered to live individuals for incubation periods of 10 days. Tritium labels were introduced at C-3 as described previously (9), and the purity of these precursors assessed by hplc and capillary gc analysis. The results of these experiments are summarized in Table 1. Interestingly, all precursors were transformed to pregnenolone [**1**], indicating that, in addition to the existence of the mammalian pathway from cholesterol, a new route from Δ^{22}-sterols also operates. We believe the route is as detailed in Scheme 3 as, in addition to the conversion of stigmasterol to pregnenolone, **8** was also transformed. Further, the pathway on the left of Scheme 3 (i.e., migration of H_a) appears to be the one operating, as radioactive **8** was recovered in the stigmasterol [**5c**] experiment.

In each case, 25% of the total pregnane sample was injected onto the hplc, and in addition to the pregnane peak, baseline fractions prior to, and following, this peak were collected and their radioactivity measured. In all cases, the radioactivity of the hplc fractions before and after the pregnane peaks was at background level (ca. 20 dpm). Also, the recovered pregnenolone [**1**] was purified to constant specific activity by hplc. The location of the tritium label was determined to be at C-3 by oxidation with pyridinium chlorochromate and measuring the radioactivity of the resulting ketone. In all cases, the products were not radioactive. Further, an aliquot of the hplc-purified pregnenolone (25% of total) was acetylated under standard conditions and purified by hplc. In all three



SCHEME 3. Pregnane biosynthesis from Δ^{22} sterols.

TABLE 1. Incorporation of Sterol Precursors in Pregnenolone [**1**].

Precursor	Radioactivity fed, dpm (recovered activity precursor)	Radioactivity recovered in  **1** (dpm)
 **5C** [3-^{3}H]	2.2×10^7 (98,970)	1,830
 **8** [3-^{3}H]	2.2×10^7 (66,053)	3,200
 **2** [3-^{3}H]	2.2×10^7 (1,002,710)	1,160

cases, the acetates showed similar activities to the purified pregnenolone [**1**].

It seems most likely that the enzymes responsible for the dealkylation of phytosterols to cholesterol are also involved in the production of pregnenolone [**1**]. Experiments are currently underway to examine precisely the mechanism and scope of pregnane biosynthesis in marine invertebrates.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Hplc was performed using a refractive index detector and reversed-phase Ultrasphere column (10 mm×25 cm i.d.), with aqueous MeOH as mobile phase. All solvents were distilled prior to use. Radioactivity was determined using a liquid-scintillation counter and a toluene-based scintillation fluid.

COLLECTION OF *AMPHIMEDON COMPRESSA* AND BIOSYNTHETIC FEEDING EXPERIMENTS.—Specimens of *A. compressa* were collected off Long Key, Florida, at a depth of ca. 20 meters. Labeled precursors **2**, **5c**, and **8** were prepared as described previously (9). Biosynthetic experiments were performed by placing a sponge in a 1000-ml beaker with sea water and adding a suspension of the labeled precursor. The sponge was allowed to filter this sea water for 6 h, rinsed with sea water, and then attached to an underwater grid on the reef. After an incubation period of 10 days the sponges were harvested, freeze-dried, and extracted in $CHCl_3$/MeOH.

PURIFICATION OF PREGNENOLONE AND STEROLS.—The sterol fraction and pregnenolone were purified from a crude extract by prep. tlc (hexane-EtOAc, 65:35). The sterols, cholesterol, stigmasterol, and pregnenolone, were further purified by reversed-phase hplc (MeOH). In each case, 25% of the total was injected. These individual sterols were shown to be homogenous by capillary gc.

PREPARATION OF 20-METHYLENEPREGNANE.—Pregnenolone acetate (25 mg) was added to a solution of the ylid generated from methyl iodide and triphenyl phosphine using standard conditions. The Wittig product was purified by reversed-phase hplc (MeOH).

OXIDATION OF PREGNENOLONE FROM FEEDING EXPERIMENT.—The hplc-purified pregnenolone from the various feeding experiments was treated with an excess of pyridinium chlorochromate in CH_2Cl_2. After 2 h, the Δ^5-ketone was recovered by passing the reaction mixture through a column of silica and evaporation of the solvent. The activities of the oxidized steroid were: 420 dpm from **2**, 750 dpm from **5c**, and 400 dpm from **8** (background activity=20 dpm).

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